File S1

SUPPORTING MATERIAL AND METHODS

Mutant alleles

ham-3(n1654)III, ham-3(tm3309)III, swsn-2.2(ok3161) I/hT2[bli-4(e937) let-?(q782) qIs48](I;III), psa-1(os22)V, psa-4(os13)IV, swsn-7(gk1041)/mIn1[mIs14 dpy-10(e128)] II, let-526(gk816) I / hT2 (I;III)

Transgenes

zdIs13: Is[tph-1::gfp], otIs266: Is[cat-1::mCherry], otIs225: Is[cat-4::gfp], otIs226: Is[bas-1::gfp], inIs179: Is[ida-1::gfp], uIs22: Is[mec-3::gfp], otIs33: Is[kal-1::gfp], otIs337: Is[unc-86 fosmid::yfp; ttx-3p::mCherry] (kind gift from Pat Gordon)*, kuIs34: Is[sem-4p::sem-4::gfp]* (kindly provided by Min Han)*,* NG2656*: Ex[ham-2::gfp; rol-6], otEx5092, otEx5142, otEx5143: Ex[ham-3 rescuing fosmid (WRM0626dF04); rol-6(d)], otEx5093, otEx5145, otEx5146: Ex[ham-3::gfp; elt-2::dsRed], otEx5094, otEx5148, otEx5149: Ex[swsn-2.2::mChOpti; ttx-3::gfp]*

Generation of transgenes

ham-3 and swsn-2.2 reporter constructs were generated by PCR fusion (HOBERT 2002). The *ham-3* genomic locus was fused to *gfp* and injected into N2 wildtype at 10 ng/μL with elt-2::dsRed at 5 ng/μL as an injection marker. The *swsn-2.2* genomic locus was fused to mChOpti (a codon-optimized version of mCherry) and injected into N2 wildtype at 5 ng/μL with *ttx-3::gfp* at 5 ng/μL as an injection marker. For rescue experiments, the fosmid WRM0626dF04 was linearized and injected at 10 ng/μL with a linearized plasmid containing *rol-6* at 5 ng/μL directly into OH9422, a strain containing the *ham-3(n1654)* mutation as well as the transgene *zdIs13*, an integrated *tph-1::gfp* reporter. All arrays were generated as complex arrays with 100-125 ng/μl of sonicated bacterial genomic DNA.

Whole Genome Sequencing

Genomic DNA was prepared from *ham-3(n1654)* mutant animals as previously described (SARIN *et al.* 2010). DNA was sequenced using a Illumina Genome Analyzer II platform and sequence analysis was done using MAQGene (BIGELOW *et al.* 2009).

RNA interference

RNAi was performed using a bacterial feeding protocol in an *nre-1 lin-15b* mutant background (SCHMITZ *et al.* 2007).

Microscopy

A Zeiss Axioplan 2 equipped with Nomarski and fluorescence optics was used. DIC and fluorescent images were collected and processed using Micro-manager (EDELSTEIN *et al.* 2010).

BIGELOW, H., M. DOITSIDOU, S. SARIN and O. HOBERT, 2009 MAQGene: software to facilitate C. elegans mutant genome sequence analysis. Nat Methods **6:** 549.

EDELSTEIN, A., N. AMODAJ, K. HOOVER, R. VALE and N. STUURMAN, 2010 Computer control of microscopes using microManager. Curr Protoc Mol Biol **Chapter 14:** Unit14 20.

HOBERT, O., 2002 PCR fusion-based approach to create reporter gene constructs for expression analysis in transgenic C. elegans. Biotechniques **32:** 728-730.

SARIN, S., V. BERTRAND, H. BIGELOW, A. BOYANOV, M. DOITSIDOU *et al.*, 2010 Analysis of multiple ethyl methanesulfonate-mutagenized caenorhabditis elegans strains by whole-genome sequencing. Genetics **185:** 417-430.

SCHMITZ, C., P. KINGE and H. HUTTER, 2007 Axon guidance genes identified in a large-scale RNAi screen using the RNAi-hypersensitive Caenorhabditis elegans strain nre-1(hd20) lin-15b(hd126). Proc Natl Acad Sci U S A **104:** 834-839.